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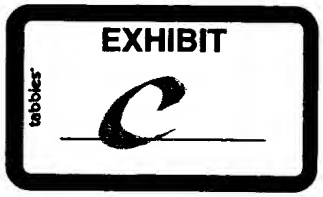

Relationship between Grain Nitrogen, Non-protein Nitrogen and Nucleic Acids during Wheat Grain Development

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Abstract

Eight wheat varieties which normally produce grain of different final percentage nitrogen content were grown under field and glasshouse conditions. The final percentage grain nitrogen of the field grown varieties ranked in the expected order; however, total nitrogen/grain, DNA/grain, RNA/grain and non-protein nitrogen/grain during grain development differed between varieties. DNA/grain reached a maximum value in all varieties between 21 and 28 days post-anthesis, suggesting a longer period of cell division than previously reported. There was no apparent relationship between final percentage grain nitrogen and either DNA, total grain RNA or the concentration of grain amino acids during development. Heads from glasshouse grown wheat were detached at 8 days post-anthesis and grown in liquid culture under conditions where the nitrogen concentration of the culture medium was varied. Fresh weight/grain, DNA/grain, RNA/grain and total grain nitrogen all increased with increasing nitrogen concentration in the culture medium, but grain dry weight remained constant at the different nitrogen concentrations. The changes in fresh weight/grain, DNA/grain and RNA/grain were not the same for all varieties. A possible relationship between total grain nitrogen and DNA/grain and RNA/grain during seed development exists for heads grown in culture for individual varieties. This apparent relationship for individual varieties cannot be used to explain intervarietal differences in total grain nitrogen because in some cases different varieties grown under identical culture conditions, although producing grain of equivalent total nitrogen, had widely differing levels of both DNA and RNA per grain.

Introduction

Grain protein percentage in wheat is determined by environment and genotype. However, little is known of how genetic control is exercised at the physiological or biochemical level. Donovan *et al.* (1977*b*), in a study on grain development using a 'high protein' variety and a 'low protein' variety, suggested a possible relationship between final percentage protein and total RNA levels per grain during development. It was suggested that higher levels of RNA/grain in the 'high protein' variety were responsible for the higher rate of protein deposition because of larger numbers of active ribosomes. However, when wheat heads of two wheat varieties, which under field conditions produce grain of very different final percentage protein, were detached at 8 days post-anthesis and grown in liquid culture (Donovan and Lee 1977, 1978) under identical nutrient regimes, the differences in percentage grain protein were very small, suggesting that control was at the source level rather than the sink level.

This paper reports experiments using eight field-grown wheat varieties varying in their final percentage grain nitrogen. Changes in composition of the grain were measured during development with the aim of relating final grain nitrogen percentage to developmental changes. Six of these varieties were also grown under glasshouse

conditions; heads were detached at 8 days post-anthesis and grown in liquid culture, in which the concentration of the nitrogenous component of the culture media was varied. The results obtained from wheat head culture experiments are compared with those obtained from studies of grain development in the intact plant.

Materials and Methods

Triticum aestivum cvv. Timgalen, SUN 9E, Timson, Gatcher, Heron, Olympic, WW15 and Egret were used. Plants were grown in adjacent single plots in the Sydney area or under glasshouse conditions. Wheat heads were labelled and harvested according to the procedure described by Donovan *et al.* (1977*a*). For estimation of dry weight, replicate samples comprising 100 grains each, taken from 10 heads, were used. These samples were subsequently pooled for chemical analyses, which were carried out in duplicate or triplicate, on composite milled grain samples. Plots were heavily fertilized wi h a mixed NPK fertilizer at planting and the plants were never allowed to suffer from water stress. Plants grown under glasshouse conditions were grown in pots in one part peat moss, one part vermiculite and one part perlite and were watered daily with a multinutrient liquid fertilizer: 15 g Aquasol (Hortico Pty Ltd). Two hundred plants of each variety were grown (five plants per pot). Heads were removed for culturing at 8 days post-anthesis. Wheat heads were cultured according to the procedure described by Donovan and Lee (1977). The following stock solutions were prepared. Major elements solution A: $CaCl_2.2H_2O$, 4·4 g, made to 500 ml. Major elements solution B: KH_2PO_4, 12·0 g, $MgSO_4.7H_2O$, 3·7 g, made to 500 ml. Minor elements solution, iron solution and vitamin solution (thiamin and myoinositol) were as described by Linsmaier and Skoog (1965). The culture media were prepared by combining the above stock solutions as follows: major elements solution A, 50 ml; major elements solution B, 50 ml; minor elements solution, 1 ml; iron solution, 2 ml; vitamin solution, 10 ml; glutamine (see results), sucrose, 40 g. The various media were adjusted to pH 5·0 and made to 1 litre.

Analytical methods for dry weight and total nitrogen were as described previously by Donovan and Lee (1977). Analytical methods for non-protein nitrogen (NPN) and RNA determination were as described by Donovan *et al.* (1977*a*, 1977*b*). Soluble RNA type III (Sigma Chemical Co., St Louis, Mo.) was used as the RNA standard. DNA was determined by estimation of 2-deoxyribose using Burton's (1956) procedure after extraction of the DNA from freeze-dried grain using the procedures described by Donovan *et al.* (1977*a*, 1977*b*); 2-deoxyribose was used as the standard. The DNA/grain was calculated by assuming that the 2-deoxyribose content of the extracted DNA was 30% and that the purine content was equal to the pyrimidine content.

Results

For the field-grown varieties, the final percentage grain nitrogen of SUN 9E (2·63% N), Timgalen (2·61% N) and Timson (2·55% N) was high, of Gatcher (2·38% N) was intermediate and of WW 15 (2·01% N), Egret (2·11% N) and Olympic (2·26% N) was low. This is in accord with their usual classification based on final percentage grain nitrogen as high, intermediate and low protein varieties.

Maximum levels of DNA/grain in field-grown varieties were not the same for all the varieties (Table 1) and there was no apparent relationship between DNA/grain and total nitrogen/grain (Table 2) or with final percentage grain nitrogen.

Non-protein nitrogen/mg dry weight of grain (Table 3) also varied from variety to variety during development. Non protein nitrogen/mg dry weight may be considered as an approximate estimate of the concentration of free amino acids available for protein synthesis. NPN concentrations at 7 days post-anthesis were higher in the high protein varieties but, by 14 days post-anthesis, these concentrations had become similar to those of the low protein varieties. Apart from the initially higher concentrations of NPN in the high protein varieties, there did not appear to be any relationship between total grain nitrogen, final percentage grain nitrogen and NPN/mg dry weight during grain development.

Table 1. DNA/grain at different stages of development for eight field-grown wheat varieties

	DNA (μg/grain)					
Time (weeks) post-anthesis:	1	2	3	4	5	6
SUN 9E	17·4	27·8	31·2	34·8	29·5	26·8
Timgalen	14·5	19·8	23·9	29·5	24·5	22·9
Timson	12·9	20·6	25·1	27·1	21·2	22·0
Gatcher	19·8	23·8	32·8	31·5	24·5	27·5
WW 15	11·3	9·4	18·4	24·4	22·2	20·6
Heron	12·7	19·9	28·9	32·9	32·3	27·0
Egret	12·5	17·8	24·1	27·1	26·3	20·0
Olympic	12·1	19·7	25·3	24·7	23·7	20·3

Table 2. Grain dry weight and total grain nitrogen at different stages of development for eight field-grown varieties

L.s.d. (5%) dry weight = 2·93

Time (weeks)	Grain dry weight (mg)						Total nitrogen (mg/grain)					
post-anthesis:	1	2	3	4	5	6	1	2	3	4	5	6
SUN 9E	6·9	17·7	31·1	40·9	46·2	46·8	0·17	0·45	0·73	0·96	1·23	1·23
Timgalen	5·4	13·8	25·4	36·8	45·7	42·7	0·13	0·30	0·56	0·82	1·11	1·11
Timson	4·9	14·3	23·5	33·7	37·3	38·8	0·11	0·32	0·54	0·87	0·93	0·98
Gatcher	8·7	17·7	34·0	44·6	49·2	51·1	0·19	0·38	0·73	0·98	1·13	1·21
WW 15	4·1	11·7	21·2	31·6	39·1	41·0	0·09	0·24	0·43	0·62	0·82	0·82
Heron	5·2	15·3	27·9	40·9	50·8	50·4	0·11	0·30	0·51	0·79	0·97	1·11
Egret	4·8	13·7	23·2	35·1	43·6	42·7	0·11	0·29	0·46	0·69	0·90	0·90
Olympic	5·0	13·4	28·0	38·7	50·5	43·3	0·11	0·26	0·51	0·69	1·01	0·98

Table 3. Non-protein nitrogen at different stages of development for eight field-grown wheat varieties

	Non-protein nitrogen (μg/mg dry weight)					
Time (weeks) post-anthesis:	1	2	3	4	5	6
SUN 9E	5·85	3·35	1·35	1·19	1·13	0·88
Timgalen	5·84	3·90	2·33	1·83	1·64	1·67
Timson	5·50	3·31	2·18	1·73	1·31	1·12
Gatcher	4·48	2·40	1·33	1·18	1·08	0·74
WW 15	4·39	3·39	2·16	1·53	1·38	1·11
Heron	3·97	2·63	2·06	1·52	1·74	1·20
Egret	4·79	3·18	2·06	1·81	1·31	0·85
Olympic	4·74	3·20	2·18	1·56	1·40	1·04

Increases in RNA/grain parallelled increases in DNA/grain, with maximum values in most cases being reached between 21 and 28 days post-anthesis (Table 4). The pattern of development varied between varieties and the maximum values reached also varied. However, there was no clear relationship between total grain nitrogen and RNA/grain during development.

Wheat heads were detached at 8 days post-anthesis and grown in liquid culture for 12 days in media containing glutamine as the nitrogen source (Donovan and Lee 1978) at concentrations of 0, 0·05 and 0·1%N. Dry weight/grain did not increase with increasing nitrogen concentration in the culture medium, consistent with a previous

Table 4. RNA/grain at different stages of development for eight field-grown wheat varieties

Time (weeks) post-anthesis:	RNA (μg/grain) 1	2	3	4	5	6
SUN 9E	68	135	135	152	139	105
Timgalen	63	163	183	153	98	91
Timson	51	120	126	144	106	68
Gatcher	71	129	157	160	106	83
WW 15	52	121	119	131	122	95
Heron	73	160	148	150	155	116
Egret	58	130	130	126	123	105
Olympic	51	121	122	121	124	88

Table 5. Total grain nitrogen, grain dry weight and grain fresh weight in heads cultured in media having different nitrogen concentrations

Time in culture (days)	Nitrogen concn in medium (%)	SUN 9E	Timgalen	Gatcher	WW 15	Heron	Egret
		Total grain nitrogen (mg/grain)					
0		0·07	0·14	0·09	0·06	0·14	0·22
6	0	0·18	0·28	0·17	0·15	0·23	0·46
	0·05	0·29	0·45	0·26	0·25	0·39	0·68
	0·10	0·35	0·47	0·34	0·30	0·51	0·61
12	0	0·29	0·43	0·21	0·28	0·33	0·47
	0·05	0·60	0·79	0·49	0·62	0·61	0·60
	0·10	0·84	0·94	0·70	0·72	0·95	0·92
		Dry weight (mg/grain)[A]					
0		2·8	5·5	3·8	2·7	5·7	7·6
6	0	9·9	14·6	11·3	9·7	14·3	22·3
	0·05	10·7	15·2	10·3	10·5	15·1	21·5
	0·10	10·4	13·4	11·1	9·7	15·2	18·8
12	0	20·8	26·8	19·1	21·2	24·1	27·9
	0·05	21·9	27·8	20·9	24·2	24·0	25·2
	0·10	22·9	25·0	19·3	21·2	25·4	26·8
		Fresh weight (mg/grain)[B]					
0		11·29	14·8	15·1	11·4	19·0	24·9
6	0	34·8	44·1	37·3	32·1	42·8	59·8
	0·05	40·3	47·6	36·7	34·6	45·4	65·5
	0·10	40·4	46·4	41·1	34·8	51·2	62·8
12	0	50·1	52·8	44·3	47·6	49·2	60·0
	0·05	60·5	63·2	49·6	56·6	52·3	58·5
	0·10	73·9	69·0	59·6	60·0	61·9	79·2

[A] L.s.d. (5%) day 0 = 0·95; l.s.d. (5%) days 6 and 12 = 3·06.

[B] L.s.d. (5%) day 0 = 2·71; l.s.d. (5%) days 6 and 12 = 7·79.

report (Donovan and Lee 1978). Fresh weight/grain, however, showed a substantial increase with increasing concentration of nitrogen in the culture medium (Table 5). DNA/grain (Table 6) also increased with increasing concentration of nitrogen in the culture medium. This increase occurred both in the endosperm and seed coat layers but not in the embryo. Because the seed coat layer was contaminated with aleurone, which is normally considered part of the endosperm, it is possible that most of the increase in DNA was in the endosperm. Maximum levels of DNA/grain were not the same for all varieties, being higher in SUN 9E, Gatcher and Timgalen and lower in Egret, WW15 and Heron. Attempts to estimate endosperm cell numbers after 12 days in culture, when the changes in DNA/grain were greatest, using the procedure described by Rijven and Wardlaw (1966), were severely hampered by the presence of large amounts of starch.

Table 6. Changes in DNA and RNA in heads cultured in media having differing nitrogen concentrations
S, SUN 9E; *T*, Timgalen; *G*, Gatcher; *W*, WW 15; *H*, Heron; *E*, Egret

Time in culture (days)	Nitrogen concn in medium (%)	DNA (μg/grain)						RNA (μg/grain)					
		S	T	G	W	H	E	S	T	G	W	H	E
0		16·4	16·0	19·4	6·9	8·2	16·6	36	83	51	38	80	93
6	0	30·2	19·6	27·6	4·9	11·5	23·9	122	128	105	94	110	118
	0·05	35·8	26·5	33·8	7·8	18·2	25·9	171	187	149	125	146	165
	0·10	36·2	27·8	37·6	10·1	18·5	25·2	173	197	172	131	193	150
12	0	32·0	26·9	27·5	9·2	10·4	23·3	111	119	81	84	74	94
	0·05	38·9	33·5	35·7	15·4	14·8	21·1	188	191	144	163	125	111
	0·10	49·1	37·7	42·2	17·0	19·8	28·7	284	227	202	195	176	138

RNA/grain, in a similar manner to DNA/grain, increased with increasing nitrogen concentration in the culture medium (Table 6). It was higher in the high protein varieties SUN 9E and Timgalen than it was in the other varieties at corresponding nitrogen concentrations. Total grain nitrogen, RNA/grain and DNA/grain all increased as the nitrogen concentration increased in the culture medium although the response was not the same for all varieties.

Analyses of the non-grain parts of the heads (rachis, rachilla and glumes) at 8 days post-anthesis and of heads which had been in culture for 12 days (Table 7) showed that percentage nitrogen fell during the course of culture as would be expected, with the largest fall being recorded for the medium in which nitrogen was omitted. Total nitrogen also fell at the two lowest culture medium nitrogen concentrations but increased at the highest nitrogen concentration in all varieties except Heron. There was no apparent relationship between total grain nitrogen and the net mobilization of nitrogen from the non-grain parts of the head.

Discussion

Maximum Grain DNA Content and Cell Division

Previous studies have suggested that cell division in the endosperm has ceased by 16 days (Evers 1970) or as late as 19 days post-anthesis (Sandstedt 1946; Jennings and Morton 1963). However, Donovan *et al.* (1977*b*) showed that increases in DNA/grain

Table 7. Changes in percentage nitrogen and total nitrogen in the non-grain parts of heads cultured in media having differing nitrogen concentrations

Each determination represents eight heads pooled, weighed and milled in a Wiley mill. *S*, SUN 9E; *T*, Timgalen; *G*, Gatcher; *W*, WW 15; *H*, Heron; *E*, Egret

Time in culture (days)	Nitrogen concn in medium (%)	N (%, dry weight basis) in head non-grain parts						Total N (mg) in head non-grain parts					
		S	T	G	W	H	E	S	T	G	W	H	E
0		2·52	2·20	1·91	2·19	1·93	2·06	10·48	6·54	5·27	4·27	7·28	9·81
12	0·0	0·60	0·60	0·70	0·59	0·55	0·68	3·58	3·29	2·74	2·12	2·49	5·22
	0·05	0·88	0·86	0·96	1·12	0·68	0·79	4·64	4·63	3·49	4·39	3·48	4·95
	0·10	1·85	1·41	1·48	1·61	1·04	1·54	11·66	7·75	6·23	6·86	5·55	14·77

began to slow between 18 and 20 days post-anthesis and that the maximum value was reached at about 25 days. Clearly however, cessation of cell division depends to a great extent on the normal development period which in turn depends on a number of environmental factors. In the present study DNA reached maximum levels between 21 and 28 days post-anthesis.

Previous studies on cessation of cell division have relied upon microscopic evidence and it may be argued that this depends heavily upon the ability of the investigators to locate mitotic figures. The chemical measurement of DNA may reflect changes other than cell division such as an increase in the DNA/nucleus or a change in the amount of extranuclear DNA. Changes in the DNA/nucleus during development in *Vicia faba* cotyledons (Millerd and Whitfield 1973) are several-fold. Maherchandani and Naylor (1971) reported deviations in nuclear DNA in *Avena fatua* aleurone cells. In a similar study carried out on developing barley aleurone, Keown (1974) showed that the cells are highly heterogeneous with respect to DNA content with the whole population of cells ranging from 2 C to 22 C. Since satellite DNA represents only a few percent of the total DNA (Beevers 1976), the increase in DNA beyond the period where, from microscopic evidence, mitosis is thought to cease probably represents either an actual increase in cell numbers or a change in the DNA/nucleus. Only an estimation of the constancy of nuclear DNA will resolve the matter.

Grain DNA Content in Intact Plants and in Cultured Wheat Heads

The DNA/grain differed between varieties in field-grown wheat. However, there was no evidence of a relationship between this parameter and fresh weight/grain, dry weight/grain, total nitrogen/grain or with final percentage grain nitrogen. This was in sharp contrast to the pattern obtained when the heads were detached at 8 days post-anthesis and grown in liquid culture. Fresh weight/grain and DNA/grain both increased with increasing nitrogen concentration in the culture medium, as also did percentage grain nitrogen. The effect of increased nitrogen supply on grain fresh weight was in agreement with previous observations in other plants where nitrogen has been reported to cause an increase in the proportion of cell water (Russell 1954). Total grain nitrogen after 12 days in culture at the three different nitrogen regimes showed some variation between the varieties. This could in part be explained by the mobilization of differing amounts of nitrogen from the non-grain part of the head during culture. Grain nitrogen, however, was largely determined by the composition

of the culture medium, especially at high nitrogen concentrations, where there was no net loss of total nitrogen from the non-grain part of the head. In a study of nitrogen metabolism in detached wheat heads in liquid culture, Lee (1978) has shown that 80–90% of the nitrogen in the non-grain parts of the head is protein nitrogen. The studies reported suggest that net mobilization of nitrogen from the grain part of the head is controlled by the nitrogen concentration in the culture medium and at high nitrogen concentrations net mobilization is totally suppressed.

The levels of DNA/grain from heads grown in culture showed considerable variation between varieties at any particular nitrogen concentration. In addition to the intervarietal variation, DNA/grain responded directly to changes in the nitrogen concentration in the culture medium. If the DNA/nucleus is constant, the results would indicate an increase in cell numbers in response to higher nitrogen concentrations in the culture medium since the contribution from plastid DNA could not account for the size of the increases observed.

Alternatively, the results may be explained by possible heterogeneity in the aleurone layers as has previously been demonstrated in the aleurone layers of normally developing *Avena fatua* (Maherchandani and Naylor 1971) and barley (Keown 1974; Taiz and Starks 1977). Because both total grain nitrogen and DNA/grain increased as nitrogen concentration increased in the culture medium, there is the suggestion of a relationship between these entities. The low protein varieties, however, produced grain of similar total nitrogen in culture to some of the high protein varieties, when the DNA/grain was in some cases as little as 40% of that of the high protein varieties. This would indicate that intervarietal differences in grain nitrogen are not related to the levels of DNA/grain.

Grain RNA Content in Intact Plants and in Cultured Wheat Heads

The levels of RNA/grain in cultured heads increased as the total nitrogen in the culture medium increased and were considerably higher after 12 days culture in the high protein varieties than in the low protein varieties. With field-grown plants, however, there did not appear to be a clear difference between the RNA contents of high and low protein varieties although the varieties showed their expected range of final percentage grain nitrogen. Approximately 60–90% of the total RNA in the developing wheat grain is ribosomal (Donovan *et al.* 1977*b*) but it is not known what proportion of this is polysomal. There are intervarietal differences in RNA/grain which occur either in field grown wheat or in cultured heads grown under identical nutrient regimes. It is not known if these differences in RNA/grain may also reflect differences in the numbers of active ribosomes. This aspect would seem to be well worth pursuing.

Non-protein Nitrogen and Protein Accumulation

Donovan *et al.* (1977*a*) reported in a study comparing the 'high protein' variety Timgalen with the 'low protein' variety Heron that NPN levels were considerably higher in the high protein variety. Calculation of the rate of protein accumulation in the grain indicated that there were about 2–3 days supply of amino acids (the principal component of non-protein nitrogen) potentially available for protein synthesis in both varieties. It might be argued that at these levels it is unlikely that the levels of non-protein nitrogen are exercising any regulatory role. In this study, although the con-

centration of NPN around 1 week post-anthesis was higher in the high protein varieties (SUN 9E, Timson and Timgalen), this difference in concentration was not sustained beyond 2 weeks after flowering. Hence it would not seem to be a major factor in determining differences in either total grain nitrogen or final percentage grain nitrogen.

In summary, it has been observed that a number of wheat varieties which, under field conditions, produce grain of different percentage protein do not show equivalent changes during development in their levels of DNA, RNA or non-protein nitrogen. When detached heads of these varieties were grown in liquid culture, all varieties produced grain of essentially the same protein percentage if grown on the same culture medium. There is evidence that, when grain protein is increased by increasing the nitrogen concentration in the culture medium, there is a corresponding increase in DNA and RNA content. It appears that, at least in the culture situation, the reasons for environmentally induced changes in grain protein content are different from those accounting for genetically determined differences. It also appears possible that control of protein content in different genotypes may be achieved in different ways.

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